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ANALYSIS OF ORGANIC PESTICIDES BY GAS CHROMATOGRAPHY

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ANALYSIS OF ORGANIC PESTICIDES BY GAS CHROMATOGRAPHY

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Introduction

Primary purpose of the grant entitled "Analysis of Organic Pesticides by Gas Chromatography" is to develop a screening program for the identification and estimation of pesticides in extracts from water and stream muds.

One phase has been a literature review which began in the summer of 1958. The file now includes more than 200 abstracts on detection techniques and related topics. Reference to the abstracts is found in Appendix A. The literature indicated that chromatographic analysis would be the best approach in developing a screening program. The literature prior to 1960 revealed that paper chromatographic and enzymatic methods were more sensitive than gas chromatographic techniques. As a result a screening program was devised, consisting of two paper chromatographic and an enzymatic method for pesticide detection.

Paper Chromatography

Chlorinated Organic Pesticides

A qualitative and semi-quantitative method, developed by Mitchell (1) and later modified by Zweig and Painter (2), was used to detect chlorinated organic pesticides. The method follows:

Apparatus and Equipment:

a. Chromatography tank and accessories

b. Whatman No. 1 filter paper (8" x 8")

c. Drying rack

d. Pyrex-glass baking dishes

e. Micro-syringe, 1 ml. capacity

f. Micro-pipettes, 5 ul., graduated in 1 ul.

g. Oven for drying papers

h. Ultraviolet light source

i. Glass plate

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1. Mitchell, L., "Separation and Identification of Chlorinated Organic Pesticides by Paper Chromatography." XI J. Assoc. Offic. Agri. Chemists, 41, 4, 71, (1958)
2. Zweig, G., and Painter, R., Private Communication (1960)

j. Goose-neck desk lamp and 60 W. bulb

k. Ruler, 12" long

l. Masking tape, 3/4" wide

Reagents:

a. Immobile solvent: 5% mineral oil (USP heavy) in ethyl ether.

b. Mobile solvent: 75% acetone in distilled water.

c. Chromogenic reagent: To 1.7 g. silver nitrate, dissolved in 5 ml. of water, add 10 ml. 2-phenoxyethanol (Eastman P4861), 1 drop of 30% H_2O_2, make up to 200 ml. with water, and add acetone until clear.

d. Sodium thiosulfate, 20% in water.

e. Standard solution of pesticides, 1 ul. = 10 ug. in ethyl acetate.

Procedure:

1. Paper must be washed with distilled water prior to chromatography. Place distilled water in stainless steel troughs in the bottom of chromatographic tank. Suspend the papers from rods and allow the water to ascend to top of the papers (may be left overnight). Use plastic gloves in handling the papers throughout the experiment.

2. Dry the papers in oven at 70° for about 30 minutes.

3. Rule line 1" from lower edge of the paper with a hard pencil. Place dots along line 1" apart.

4. Below each dot, pencil-in the code for the sample to be spotted on that dot.

5. Tape the paper to the glass plate so that the line with dots extends over the edge.

6. Bring the lamp close to paper. (Heat from the lamp will speed drying of the spots).

7. Attach a pipette to the syringe with a short rubber tube. <u>For Standards</u> Draw a known amount of the standard into lambda pipette. Hold the tip of the pipette over the penciled dot on the paper and allow a small amount of

sample (about 1 ul.) to touch the paper. As the spot dries, gradually add more until 1-5 ul. of standard is on the paper. Keep the spot as small as possible.

For Samples To the dry sample in a conical centrifuge tube add 1 or 2 drops petroleum ether to dissolve the sample. Draw sample up into lambda pipette and spot in the same manner as the standards, keeping the spot as small as possible. Repeat until all the ether solution has been spotted and then rinse the tube twice more with 1 or 2 drops of ether, spotting as before.

8. Remove the papers from the glass plate and immerse in a dish containing the immobile solvent. Immerse from top down just to the line of spots and remove immediately.

9. Suspend the papers on rods by the bottoms and allow to air-dry.

10. To each trough in the bottom of the chromatographic tank add 50 ml. mobile solvent (75% acetone). Hang the papers right side up from rods, supported by clamps so that the lower edges are immersed in mobile solvent. Place the glass cover on top of the tank and seal with masking tape. The atmosphere will become saturated with mobile solvent. Allow solvent front to ascend for 1 1/2 hours but do not let it reach a front higher than 2" from the top of the paper.

11. Remove the papers from the tank and allow them to air-dry for 10 minutes.

12. Dip the papers into silver nitrate (chromogenic reagent).

13. Place them in oven (70°C) for 10 minutes.

14. Place the papers under ultraviolet light 1 minute on each side and then 15 minutes on each side.

15. If the papers are to be preserved, follow these procedures:

    a. Wash the papers in acetone (in pyrex dish) and allow them to air-dry.

    b. Wash the papers in thiosulfate (in pyrex dish) and rinse in distilled water.

    c. Hang up the papers to dry.

    d. Mark the spots and read them in densitometer.

Calculations:

1. Qualitative

$$R_f \text{ value} = \frac{\text{distance pesticide spot moves from origin}}{\text{distance solvent moves from the origin}}$$

2. Quantitative

The concentration of the pesticide is proportional to the product of the area of the spot times the densitometer reading.

The R_f value is a unique property of each pesticide, and can be used for identification. To facilitate the identification, the R_f values of known chlorinated organic pesticides must be determined. Table I lists the R_f values of 32 chlorinated organic insecticides at various concentrations, and Table II lists the R_f values of 16 chlorinated organic herbicides.

This technique detected DDT in samples containing as low as 0.5 microgram, while for most other chlorinated organic pesticides the minimum detectable quantity was 2 micrograms. This method had limitations: (a) many chlorinated pesticides had nearly identical R_f values; (b) some pesticides appeared as streaks rather than spots, and (c) a few chlorinated organic pesticides did not respond. However, by limiting the size of the sample and by applying the sample carefully to the paper certain objections were eliminated.

The above method was further evaluated using field samples which had been processed through a column of florosil to remove much of the extraneous matter. Enough extraneous substances remained to streak the chromatogram as shown in figure 1.

The foregoing is a typical chromatogram from field samples. It is apparent that interpretation of this type of chromatogram is virtually impossible.

Pesticides were collected from water by the method described by Middleton and Rosen (4). The technique utilized the adsorption properties of carbon for removing the pesticides from the water. Pesticides were deadsorbed from the carbon by extraction with a volatile organic solvent, e.g. n-hexane or petroleum ether. The solvent was evaporated or distilled, leaving a pesticide residue. Before chromatographic analysis could be performed the pesticide residues were taken up in an organic solvent and sent through a "clean-up" column to remove extraneous organic matter. Prior to the 1962 period, Florosil was used as the adsorbant.

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3. Mitchell, L., "Separation and Identification of Eleven Organophosphate Insecticides by Paper Chromatography", J. Assoc. Offic. Agri. Chemists 43, 812, (1960)
4. Rosen, A. A., and Middleton, F. M., "Chlorinated Insecticides in Surface Water, Anal. Chem. 31, 1729, (1959)

Table I. R_f values of samples of pesticides for an aqueous system with silver nitrate : 2-phenoxyethanol in acetone as chromogenic agent

Sample No.	Temperature °C	Order of Intensity of Spot	Aqueous Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
Aldrin	20	1	0.26 ± .06	0.26 ± .06	0.26 ± 0.06	0.27 ± 0.06	0.26 ± .06
Aramite	20	1	F	F	F	F	F
BHC high	20	1	0.70 ± 0.1	0.61 - .80	0.58 - .84	.52 - .88	.50 - .91
BHC low	20	1	0.70 ± 0.1	0.71 ± 0.1	.63 - .83	.54 - .86	.54 - .91
Captan	20	1	---	---	.62 - .72	.76 - .90	.51 - .96
Chlordane	20	1	---	0.29 - .44	0.30 - .49	.27 - .52	0.26 - .54
Chlorobenzilate	20	1	---	0.84 - .99	0.83 - .96	0.82 - .99	0.77 - .99
DDD	20	1	0.69 ± .06	0.69 ± .06	0.68 ± 0.06	0.68 ± 0.06	0.68 ± .06
DDD	20	2	---	---	---	0.45 ± .06	0.48 ± .06
DDT Tech.	20	1	0.40 ± .06	0.40 ± .06	0.40 ± 0.06	0.40 ± .06	0.40 ± .06
DDT Wet	20	1	---	.41 ± .03	0.42 ± 0.4	0.43 ± .05	0.43 ± .05
Dibrom	20	1	1.0	1.0	1.0	1.0	1.0
Dieldrin	20	1	0.51 ± 0.09	0.49 ± 0.09	0.51 ± 0.1	0.50 ± 0.09	0.50 ± 0.09
Dipterex	20	1	---	---	---	---	---
Dyrene	20	1	F	F	F	F	F
Endrin	20	1	.47 ± .03	.49 ± .06	.48 ± .03	.49 ± 0.04	.49 ± 0.04

Table I Continued. R_f values of samples of pesticides for an aqueous system with silver nitrate : 2-phenoxyethanol in acetone as chromogenic agent

Sample No.	Temperature °C	Order of Intensity of Spot	Aqueous Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
Fenson	20	1	F	F	F	F	F
GC - 2466	20	1	F	F	F	F	F
Genite	20	1	F	F	F	F	F
Heptachlor	20	1	0.40 ± .09	0.40 ± 0.08	0.41 ± .06	0.38 ± 0.10	0.39 ± 0.01
Kelthane	20	1	0.70 ± .08	0.72 ± 0.1	0.73 ± .07	0.73 ± 0.08	0.72 ± 0.06
Kepone	20	1	F	F	F	F	F
Korlan	20	1	0.69 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	.69 ± 0.12
Lindane	20	1	0.70 ± 0.09	0.71 ± .07	0.72 ± .07	0.72 ± 0.08	0.72 ± 0.06
Methoxychlor	20	1	0.77 ± 0.15	0.76 ± 0.23	0.76 ± .15	0.77 ± 0.15	0.77 ± 0.15
Ovex	20	1	0.83 - .99	0.83 - .99	0.81 - 1.00	.82 - 1.00	.81 - 1.00
Perthane	20	1	---	---	---	---	---
Phygon	20	1	0.94 ± .06	0.89 - 0.99	0.81 - 0.97	0.71 - 0.98	0.49 - 0.98
Spergon	20	1	0.74 - .98	0.73 - 0.98	0.63 - 0.98	0.44 - 0.98	0.10 - 0.98
Tedion	20	1	0.72 ± 0.12	0.73 ± 0.15	0.74 ± 0.10	0.73 ± 0.12	0.72 ± 0.17
Terrachlor	20	1	---	0.41 ± 0.05	0.39 ± 0.09	0.38 ± 0.11	0.38 ± 0.11
Thiodan	20	1	0.52 ± 0.03	0.54 ± .04	0.55 ± 0.05	0.55 ± 0.07	0.56 ± 0.1

Table I Continued. R_f values of samples of pesticides for an aqueous system with silver nitrate : 2-phenoxyethanol in acetone as chromogenic agent

Sample No.	Temperature °C	Order of Intensity of Spot	Aqueous Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
Thiodan	20	2	0.69 ± .06	0.80 ± 0.08	0.81 ± 0.1	0.82 ± 0.07	0.83 ± 0.10
Toxaphene	20	1	0.30 - .54	0.30 - 0.53	0.25 - 0.57	0.24 - 0.60	0.22 - 0.61

Note: F indicates fluorescences at the solvent front

Table II R_f values of samples of pesticides for an aqueous system with silver nitrate : 2-phenoxyethanol in acetone as chromogenic agent

Sample No.	Temperature °C	Order of Intensity of Spot	Aqueous Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
Atrazine	20	1	F	F	F	F	F
Butyrac	20	1	---	---	---	---	---
Diuron	20	1	1.0	1.0	1.0	1.0	1.0
GC - 6499	20	1	1.0	1.0	1.0	1.0	1.0
GC - 6689	20	1	---	---	---	---	---
GC - 6690	20	1	---	1.0	1.0	1.0	1.0
GC - 6691	20	1	1.0	1.0	1.0	1.0	1.0
Neburon	20	1	1.0	1.0	1.0	1.0	1.0
NIA - 5996	20	1	---	1.0	0.91 - 1.00	9.89 - 1.00	0.85 - 1.00
Telvar	20	1	1.0	1.0	1.0	1.0	1.0
2,4-D Acid	20	1	0.92 ± 0.04	0.91 ± 0.05	0.91 ± 0.05	0.91 ± 0.05	0.91 ± 0.4
2,4-D Butyl	20	1	1.0	1.0	1.0	1.0	1.0
2,4-D Amine	20	1	0.94 ± 0.05	0.94 ± 0.05	0.92 ± 0.03	0.92 ± 0.05	0.92 ± 0.05
2,4-D Iso-octyl	20	1	0.63 ± 0.1	0.63 ± 0.07	0.62 ± 0.07	0.61 ± 0.06	0.61 ± 0.10
2,4-D Iso-octyl	20	2	---	---	---	---	0.36 ± 0.10
2,4-D Iso-propyl	20	1	0.87 - 1.0	0.86 - 1.0	0.86 - 1.0	.84 - 1.0	0.82 - 1.0

Table II Continued. R_f values of samples of pesticides for an aqueous system with silver nitrate : 2-phenoxyethanol in acetone as chromogenic agent

Sample No.	Temperature °C	Order of Intensity of Spot	Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) Aqueous 2	5	10	20	50
2,4,5-T Acid	20	1	0.95 ± 0.05	0.94 ± 0.06	0.94 ± 0.06	0.94 ± 0.06	0.78 - 1.0
2,4,5,-T Iso-octyl	20	1	0.50 ± 0.06	0.50 ± 0.08	0.50 ± 0.05	0.49 ± 0.05	0.49 ± 0.08
2,4,5,-T Iso-propyl	20	1	0.97 ± 0.03	0.97 ± .03	0.95 ± 0.05	0.92 ± .10	0.68 - 1.00
Urab	20	1	0.98 ± 0.01	0.98 ± 0.02	0.98 ± 0.02	0.97 ± 0.03	0.96 ± 0.04
Urox	20	1	0.98 - 1.00	0.96 - 1.00	0.94 - 1.00	0.91 - 1.00	0.90 - 1.00
Zobar	20	1	0.94 ± 0.08	0.93 ± 0.04	0.92 ± .04	0.91 ± 0.07	0.91 ± 0.07

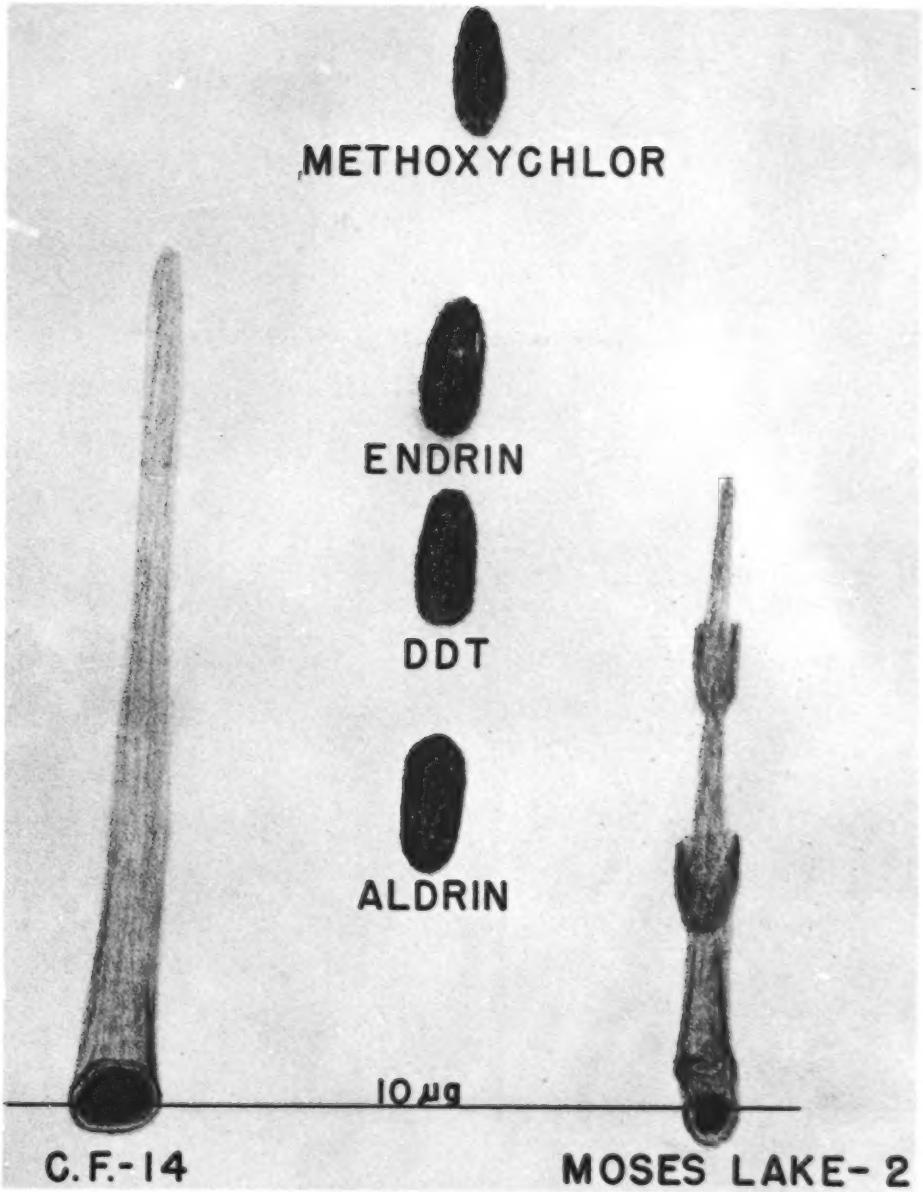

Fig. 1 PAPER CHROMATOGRAM
OF C.F.-14 AND MOSES LAKE-2

Organo Phosphate Pesticides

A paper chromatographic method developed by Mitchell (3) and modified by the principal investigator was used to separate and identify phosphoorganic pesticides. The method is as follows:

Equipment:

(a) Chromatography tank and accessories such as the Thomas-Mitchell assembly, A. H. Thomas Cat. No. 3677.

(b) Ultra-violet lamps. Model C-3 Chromato-Vue, equipped with shortwave and longwave lamps or two 15 W. germicidal lamps.

(c) Dipping tank and accessories A. H. Thomas Cat. No. 3680D or Pyrex glass trays 9" x 17".

(d) Drying rack, A. H. Thomas Cat. No. 3680F.

(e) Micro-syringe 1 ml. Hamilton Co. Cat. No. 0.0010 or equivalent.

(f) Micro-pipettes 5 ul. graduated in 1 ul.

(g) Glass plates approx. 11" x 20"

(h) Goose-neck desk lamps.

(i) Chromatographic paper - Whatman filter paper No. 1, 8" x 8" sheets.

(j) Chromatographic spray bottle.

Reagents:

(a) Bromine ACS grade

(b) Fluorescein

(c) N, N-dimethylformamide (DMF)

Standards:

Solution A: Dissolve 0.1 ml if a liquid or 0.1 g. if a solid of each organic phosphate pesticide in a 10 ml. glass stoppered volumetric flask in ethyl acetate, and dilute with ethyl acetate to 10 ml. Thus 1 ul. = 10 ug. or 10 ul. of pesticide.

Solution B: Dilute 1 ml. of Solution A to 10 ml. of ethyl acetate, 1 ul. = 1 ug. or 1 ul. of pesticide.

Immobile Solvent: Dilute 50 ml. of USP heavy mineral oil with ethyl ether to 500 ml.

Mobile Solvent: Dilute 50 ml. of DMF (N, N-dimethylformamide) with water to 100 ml. and mix.

Chromogenic Reagent:

(1) Bromine ACS grade.

(2) 0.25% (W/V), fluorescein in N, N-dimethylformamide (DMF) stock solution.

(3) Dilute 2 ml. of the stock solution to 200 ml. with 95% ethanol (spray solution).

Procedure: Aqueous Solvent System - One Dimensional

(a) Papers must be washed with distilled water and dried before use.

(b) Pipette aliquots of the extracts into 15 ml. centrifuge tubes. Evaporate to dryness using a stream of cool air (45 deg. C.) Redissolve the residue in 1 - 2 drops of ethyl ether or petroleum ether.

(c) Rule a line 1" from lower edge of paper with a hard pencil. Place dots along the line 1" apart.

(d) Below each dot, pencil-in the code for the sample to be spotted on that dot.

(e) Insert the paper between two glass plates so that the line with dots extends over the edge.

(f) Bring the lamp close to paper. (Lamp heat will hasten drying of the spots).

(g) Attach pipettes to syringe with a short piece of rubber tubing. Spot the standards and samples on the paper, taking care to keep the spots small. Never spot more than 2 ul. at one time. Dry the spots and apply another 2 ul.

(h) Transfer the papers from the glass plates to a dipping tank or tray containing immobile solvent. Immerse from the top of the paper down just to line of the spots, then remove immediately.

(i) After the papers have been dipped, clip them to the rods, and dry for a few minutes. Add 50 ml. of the mobile solvent to each trough. Hang the papers right side up from the rods, so that the lower edges are immersed in the mobile solvent. Place glass cover on top of tank and seal with masking tape. Allow solvent front to ascend from 1 1/2 to 2 hrs. Front should not be allowed to ascend any higher than 2" from top of the paper.

(j) Remove the papers and allow them to air-dry overnight in moving air.

(k) Pipette a small quantity of Bromine into a wide-mouth gallon jar and cover (1 ml. Br. will fune 16-20 chromatograms).

(l) Roll the chromatograms, place them in a holder, and place the chromatogram in jar, cover, and fume about 30 seconds.

(m) Uncover the jar and remove the chromatograms within 30 seconds. Spray lightly, (just dampen) with dilute fluorescein for 1 to 1 1/2 min. at pressure of 3-5 psi. Within 3-5 minutes view the papers under shortwave ultraviolet light. Circle the quenched spots with a soft pencil. Under shortwave light, the pesticides appear as quenched areas (deep, dark blue or black) against a greenish yellow background. Under visible light there is no color contrast.

Calculations:

$$R_f \text{ value} = \frac{\text{distance the pesticide spot moves from the origin}}{\text{distance the solvent moves from the origin}}$$

Organic phosphate pesticides consisting of isomers showed a spot for each constituent. The method's one serious limitation was that contrast time between the background and quenched areas began to dissipate after 3 minutes. This method had no quantitative value, but qualitatively it had a sensitivity of 5 micrograms. Difficulties in the interpretation of the chromatograms were not encountered when "Cleaned" field sample concentrates were used.

The R_f values were determined for 18 common organic phosphate pesticides. This information appears in Table III.

Table III R_f values of samples of pesticides for an aqueous system with fluorescein and N, N-dimethylformamide in ethyl alcohol as chromogenic agent

Sample No.	Temperature °C	Order of Intensity of Spot	Aqueous Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
Delnav	20	primary	0.30 ± 0.08	0.30 ± 0.07	0.30 ± 0.10	0.30 ± .10	0.30 ± .10
Diazinon	20	primary	0.33 ± .04	0.33 ± .05	0.33 ± .09	0.32 ± 0.1	0.31 ± 0.1
Dibrom	20	primary	0.90 ± .08	0.90 ± .05	0.90 ± 0.07	0.89 ± 0.04	0.87 ± 0.07
Dipterex	20	primary	---	---	---	---	---
Disyston	20	primary	0.19 ± 0.05	0.20 ± 0.06	0.19 ± .03	0.18 ± .03	0.18 ± .04
Disyston	20	secondary	---	---	0.41 ± 0.04	0.41 ± 0.04	0.41 ± 0.04
EPN	20	primary	0.33 ± 0.04	0.32 ± 0.06	0.32 ± 0.04	0.31 ± 0.07	0.29 ± .07
EPN	20	secondary	---	---	---	.93 ± 0.02	0.93 ± .03
Ethion	20	primary	0.14 ± 0.03	0.14 ± 0.02	0.14 ± 0.02	0.14 ± .02	0.12 ± 0.06
Ethion	20	secondary	---	---	0.87 ± 0.04	0.81 - 1.00	0.81 - 1.00
Guthion	20	primary	0.87 ± 0.05	0.88 ± 0.06	0.84 ± 0.07	0.85 ± 0.06	0.82 ± 0.09
Malathion	20	primary	0.79 ± 0.06	0.80 ± 0.08	0.79 ± 0.08	0.77 ± 0.08	0.77 ± 0.08
Methyl Parathion	20	primary	0.79 ± 0.05	0.78 ± 0.05	0.78 ± 0.05	0.77 ± 0.06	0.75 ± 0.06
Methyl Parathion	20	secondary	0.94 ± 0.03	0.97 ± 0.03	0.96 ± 0.04	0.95 ± 0.05	0.94 ± 0.06
OMPA	20	primary	---	---	0.96 ± 0.04	0.94 ± 0.03	0.91 ± 0.03
Parathion	20	primary	0.47 ± 0.05	0.47 ± 0.05	0.50 ± 0.08	0.48 ± 0.09	0.47 ± 0.1

Table III Continued. R_f values of samples of pesticides for an aqueous system with fluorescein and N, N-dimethylformamide in ethyl alcohol as chromogenic agent

Sample No.	Temperature °C	Order of Intensity of Spot	Aqueous Quantity of Substance in Micrograms (Solids) or Microliters (Liquids) 2	5	10	20	50
Parathion	20	secondary	---	0.96 ± 0.1	0.97 ± 0.03	0.97 ± 0.05	0.97 ± 0.05
Phosdrin	20	primary	0.95 ± .02	0.96 ± 0.02	0.96 ± 0.02	0.95 ± 0.02	0.94 ± 0.02
Phostex	20	primary	0.05 - 1.00	0.05 - 1.00	0.05 - 1.00	0.05 - 1.00	0.05 - 1.00
Ronnel	20	primary	0.18 ± 0.03	0.17 ± 0.03	0.16 ± 0.04	0.17 ± 0.03	0.16 ± .03
Ronnel	20	secondary	---	---	0.28 - .51	0.30 - 0.60	0.27 - 0.61
Systox	20	primary	0.90 ± 0.05	0.89 ± 0.03	0.89 ± 0.05	0.89 ± 0.04	0.88 ± 0.05
Systox	20	secondary	0.29 ± 0.06	0.30 ± 0.03	0.29 ± 0.05	0.29 ± 0.05	0.28 ± 0.03
Tepp	20	primary	0.99 ± 0.01	0.98 ± 0.02	0.97 ± 0.02	0.95 ± 0.02	0.93 ± 0.04
Thimet	20	primary	0.23 ± 0.03	0.23 ± 0.03	0.21 ± 0.03	0.22 ± 0.03	0.20 ± 0.03
Thimet	20	secondary	---	---	0.94 ± 0.02	0.94 ± 0.02	0.93 ± 1.0

Enzymatic Analysis

Another approach was taken to obtain information of quantitative significance of samples containing organic phosphate pesticides. The quantity of organic phosphate pesticides was determined by a modification of the enzymatic method of Giang (2) (5). This method depended upon the degree of deactivation of the enzyme acetylcholinesterase. Thus the degree of deactivation was indicative of the gross amount of phospho-organic pesticides present. The method is as follows:

Equipment and Supplies:

(a) Miscellaneous volumetric pipettes (1, 3, 5, 10 mls.)

(b) 10 ml. Griffin beakers

(c) Glass or stainless steel rods to fit into the bottom of the beakers.

(d) Dryer

(e) Shaker incubator

(f) pH meter with microelectrodes

(g) Electric timer or stopwatch

Reagent:

(a) Glycerol

(b) Methanol

(c) Horse plasma

(d) Sodium barbital

(e) Potassium dihydrogen phosphate

(f) Acetylcholine bromide

(g) Sodium chloride, Potassium Chloride

Solutions:

Glycerol-methanol: 10 ml. of glycerol is made up to 100 mls. with absolute methanol.

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5. Giang, P. A., "Enzymatic Determination of Organic Phosphorus Insecticides. Anal. Chem. 23, 1830, (1951)

Barbital buffer: 7.42 g. sodium barbital, 89.46 g. potassium chloride and 1.09 g. potassium dihydrogen phosphate are added to 900 mls. of distilled water. Adjust the pH to 8.00 ± 0.05 with 0.1 N hydrochloric acid and make up to one liter. This solution must be refrigerated.

Saline: 0.9% sodium chloride in distilled water.

Acetylcholinesterase solution: 4 volumes of plasma, 2 volumes saline solution and 10 volumes of buffer are combined. This solution must be made up fresh daily and refrigerated when not in use.

Acetylcholine bromide: 4.0 g. of acetylcholine bromide are dissolved in 100 mls. of distilled water. The solution must be stored in a refrigerator.

Procedure:

(a) Pipet 0.1 and 1.0 ml. aliquots of the residue redissolved in benzene into 10 ml. beakers. Suitable blanks are prepared at this time. Run all samples in duplicate.

(b) Add 0.5 ml. of the glycerol-methanol solution to each beaker.

(c) Evaporate the benzene and methanol with a stream of cool air from an air blower.

(d) Add a stainless steel rod to each beaker.

(e) Add 3.0 mls. of acetyl cholinesterase solution to each beaker at three minute intervals.

(f) Agitate each beaker in the shaker incubator at 25°C for 30 minutes.

(g) Determine the pH of each beaker, starting about 20 minutes after the addition of the acetylcholinesterase solution. This pH is referred to as the initial pH. This operation does not have to be timed.

(h) Add 1.0 ml. acetylcholine bromide solution to each beaker exactly 30 minutes after addition of the enzyme (acetylcholinesterase).

(i) Read the final pH of each beaker exactly 60 minutes after the addition of the substrate (acetylcholine).

Calculations:

$$pH = \text{initial pH} - \text{final pH}$$

$$\% \text{ inhibition} = \frac{\text{pH of blank} - \text{pH sample}}{\text{pH of blank}} \times 100$$

Zweig and Painter (2) used pseudo-oxidation with peracetic to increase the inhibition activity of the phosphothioates and dithioates pesticides. Our investigations found pseudo-oxidation decreased the activity of the phosphorate, pyrophosphate and phosphate pesticides but did not affect the activity of the carbamate and a few partially oxidized phosphothioates.

Inhibition studies, using both oxidized and unoxidized samples, obtained not only quantitative information but also some information regarding the chemical structure of the compound present.

The Cholinesterase inhibition data for enzyme inhibiting pesticides is given in Table IV.

The inhibition-concentration relationship was used to determine quantity of cholinesterase inhibiting substance if the identity of that substance was known. Where two or more inhibiting compounds were present, the quantity of each could not be accurately determined.

While studies using paper chromatography and enzymatic analysis were underway, investigations were being conducted to find a "clean-up" agent more efficient than Florosil. After testing a number of substances, it was learned from Dr. Coulson of Stanford Research Institute that by activating aluminum silicate at 300°C a highly efficient clean-up substance could be attained (6). Extraneous matter in the elutants of field samples passed through a column of activated aluminum silicate did not cause interference when the sample was analyzed for chlorinated organic pesticides by paper chromatography.

This screen program while satisfactory, had two disadvantages: it was slow and at best, semi-quantitative.

Gas Chromatography

Research activities were directed towards the gas chromatographic analysis of pesticides. Coulson, et. al (6) described a method for the determination of pesticide residues in fresh vegetables by gas chromatography. Coulson et. al (6) found that a gas chromatographic column system consisting of 30% (by weight) of Dow 11 silicone grease on 60/80 mesh chromosorb at 250°C gave good separation of pesticides singly or in mixtures.

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6. Coulson, D. M., and Devries, J. E., "Pesticide Residues on Fresh Vegetables." Stanford Research Institute Report 6, II, 1958.

Table IV

Concentration of Pesticides to Produce % Inhibition of Acetylcholinesterase

Compound	Oxidized	% Inhibition Micrograms							
		10	20	30	40	50	60	70	80
Delnav	no	0.19	0.34	0.50	0.66	0.83	1.25	1.70	2.40
Delnav	yes	0.0036	0.006	0.01	0.014	0.021	0.027	0.034	0.045
Diazinon	no	11	18.5	31	44	60	87	210	600
Diazinon	yes	--	0.017	0.031	0.043	0.062	0.079	0.101	0.130
Dibrom	no	--	--	1.1×10^{-5}	1.5×10^{-5}	2×10^{-5}	2.8×10^{-5}	3.8×10^{-5}	5×10^{-5}
Dibrom	yes	0.007	0.01	0.015	0.022	0.031	0.044	0.066	0.10
Dipterex	no	0.29	0.56	0.82	1.2	1.65	2.2	3.0	4.2
Dipterex	yes	17.0	42.0	70.0	100	140	--	--	--
Disyston	yes	0.54	1.13	1.70	2.30	3.30	4.5	6.5	8.1
EPN	yes	0.13	0.26	0.50	0.60	0.71	0.84	0.95	1.05
EPN	no	No inhibitions in concentrations less than 10 mg.							
Ethion	yes	0.0051	.0074	0.0105	0.17	0.25	0.38	0.53	0.62
Guthion	yes	--	0.009	0.053	0.13	0.175	0.27	0.40	0.60
Malathion	yes	--	9.0	17	30	42	60	90	--
Methyl parathion	yes	0.27	0.59	0.92	1.25	1.70	2.20	3.3	5.0
OMPA	yes/no	No inhibition in concentrations less than 100 mg.							
Parathion	yes	0.074	0.123	0.172	0.24	0.33	0.43	0.56	0.73

Table IV Continued

Compound	Oxidized	10	20	30	40	50	60	70	80
Phosdrin	no	0.014	0.0225	0.037	0.059	0.083	0.11	0.14	0.30
Phosdrin	yes	0.07	0.123	0.190	0.30	0.44	0.57	0.75	1.95
Phostex	yes	0.043	0.080	0.103	0.195	0.28	0.40	0.53	0.70
Ronnel	no	0.075	0.096	0.13	0.18	0.26	0.40	0.65	0.94
Systox	no	0.90	1.25	1.65	2.15	2.8	4.2	6.5	10.0
Systox	yes	0.95	1.85	2.75	4.0	5.10	7.45	10.0	13.0
Tepp	no	3.2×10^{-4}	7×10^{-4}	10×10^{-4}	14.5×10^{-4}	20.3×10^{-4}	29×10^{-4}	40×10^{-4}	60×10^{-4}
Thimet	yes	0.046	0.062	0.084	0.115	0.160	0.212	0.29	0.38
Sevin	no	0.24	0.38	0.60	0.97	1.25	2.10	3.0	6.60
Sevin	yes	0.46	0.61	0.81	1.06	1.40	2.12	3.0	4.80

A 1/4" stainless steel tube 6 ft. long, packed with 30% Dow 11 silicone grease on 60/80 mesh Chromosorb, was installed in a Beckman GC-2 Gas Chromatograph equipped with a thermo-conductivity detector. Pesticide standards were prepared containing 25 mg. of active ingredient per 1 ml of benzene for each pesticide listed in Tables I, II, and III. The chromatographic conditions maintained in determining the retention time for each pesticide were: column temperature 247°C, carrier gas-helium, carrier gas flow range 120 mls/min., current applied to the detector 250 milliamperes and an attenuation 2.

Table V is a list of retention times obtained using the above conditions.

From the retention time data it is evident that positive identification can not be based solely on retention time. This is especially true with multi-peak components. For example, if on a chromatogram a peak appears at 22.0 minutes it can be attributed to chlorobenzilate, the third peak of DDT or the third peak of 2,4-D iso-octyl ester. Numerous overlapping retention times can be observed. Another objection is the relative insensitivity of the detector. The thermal conductivity detector measured component pesticides in quantities of 250 micrograms or greater. The sensitivity of the paper chromatographic method was 100 times as great as the gas chromatographic method using a thermal conductivity detector. With the advent of the hydrogen flame ionization detector gas chromatography had a detection sensitivity equal to or greater than paper chromatographic methods.

Quantitative information can be obtained from the area under peak-concentrations or the peak height-concentration relationship. A 50 ug. sample of aldrin gave a peak height of 11 cm., while a 25 ug sample gave a 5.5 cm. peak, and a 12.5 ug sample gave a peak height of 2.3 cm. Thus a linear relationship exists between concentration and peak height.

The one serious limitation of gas chromatography utilizing silicone oil or grease (similar elution times of many chlorinated organic and phospho-organic pesticides) was overcome by adding Epon 1001 (an epoxy resin) and decreasing the silicone grease or oil content in the liquid phase. This modification had been suggested by the work of Goodwin, et. al. (7).

Several pesticides were re-examined using the following conditions: a 6 ft., 1/4" stainless steel column packed with 10% (by weight) of Dow 11 and 2% (by weight) Epon 1001 on 60/80 mesh acid-washed Chromosorb W, column temperature 247°C, carrier gas helium at a flow rate of 120 mls./min. and a 250 milliamperes applied to the detector. Table VI lists retention times of certain compounds.

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6. Goodwin, E. S., Goulden, R., and Reynolds, J. G., "Rapid Identification and Determination of Residues of Chlorinated Pesticides in Crops by Gas-liquid Chromatography." The Analyst 6, 1028, 677, 1961.

TABLE V
Retention Data

Compound	Amount Injected (ug.)	Peak Nos.	Retention Time (min.)
Aldrin	12.5	1	12.8
Aramite	25	1,2	2.5, 5.3
BHC high gamma	12.5	1,2	5.7, 6.5
BHC low gamma	12.5	1,2	5.4, 6.5
Captan	25	1	3.5
Chlordane	25	1,2	10, 12.3
Chlorobenzilate	25	1	22.9
DDD	25	1	27.0
DDT (Tech.)	25	1,2,3	15.3, 21.9, 26.9
DDT (Wettable)	25	1,2,3	16, 20.4, 26.6
Dibrom	12.5	1	1.6
Dieldrin	12.5	1	21.8
Dipterex	12.5	---	Decomposes
Dyrene	12.5	1	13.3
Endrin	12.5	1,2	25.4, 36.3
Fenson	12.5	1	11.8
GC - 2466	12.5	1	8.8
Genite	12.5	1,2,3,4,5	2.3, 2.8, 6.7, 11.3, 18.6
Heptachlor	12.5	1	11.0
Kelthane	12.5	1,2	1.7, 13.0
Kepone	12.5	1	31.1
Korlan	12.5	--	--
Lindane	12.5	1	6.8
Methoxychlor	25	1,2	29.3, 40.5
Mitox	12.5	1	17.1

Table V Continued

Compound	Amount Injected (ug.)	Peak Nos.	Retention Time (min.)
Ovex	25	1,2	4.5, 18.2
Perthane	25	1	24.0
Phygon	25	1	4.5, 7.2
Spergon	25	1	8.8
Tedion	25	---	None
Terrachlor	25	1	6.8
Thiodan	12.5	1,2	19.3, 25.2
Toxaphene	12.5	---	None
Atrazene	25	1	5.2
Butyrac	50	---	None
Diuron	---	---	---
GC - 6499	50	1	2.2
GC - 6689	---	---	None
GC - 6690	50	1	1.3
GC - 6691	50	---	2.0
Neburon	---	---	---
NIA - 5996	25	1	2.0
Telvar	25	---	None
2,4-D acid	25	1	None
2,4-D - Butylester	25	1,2,3	1.7, 2.3, 7.5
2,4-D - Methylamine	25	---	None
2,4-D - iso octylester	25	1,2,3	2.1, 19.7, 22.4
2,4-D - isopropylester	50	1,2,3	3.8, 5.7, 8.0
2,4,5-T Iso octyl	50	1,2,3	1.7, 3.2, 34.9

Table V Continued

Compound	Amount Injected (ug.)	Peak Nos.	Retention Time (min.)
2,4,5-T Isopropyl	50	---	None
Urab	50	---	None
Urox	50	1	2.1
Zobar	50	---	None
Delnav	50	1,2,3,4	1.1, 2.2, 3.3, 7.0
Diazinon	50	1,2	2.7, 3.3, 7.0
Dibron	12.5	1	1.6
Dipterex	---	---	Decomposes
Disyston	50	1,2	1.3, 7.8
EPN	25	1,2,3	1.0, 2.6, 4.0
Guthion	25	---	None
Malathion	25	1	2.3, 11.7
Methyl Parathion	25	---	Decomposes
OMPA	25	1,2	1.3, 6.6
Parathion	50	1,2	5.5, 13.5
Phosdrin	50	1	1.4
Phostex	50	1,2	0.9, 2.0
Ronnel	50	1,2	2.5, 12.5
Systox	50	1,2	1.3, 6.1
TEPP	50	1,2	1.0, 3.8
Thimet	---	---	---

Table VI
Retention Data

Compound	Peak No.	Retention Time (min.)
Aldrin	1	4.1
BHC High gamma	1,2	1.9, 2.2
Chlordane	1,2,3	2.7, 3.4, 5.3
DDD	1,2	6.6, 8.6
DDT	1,2	8.6, 10.6
Endrin	1	7.8
Heptachlor	1	3.3
Lindane	1	2.4
Methoxychlor	1,2	12.5 and 15.8
Ovex	1	7.2
2,4-D butylester	1	2.4
Delnav	1	2.1
Diazinon	1	1.7
Dipterex	---	Present in carrier solvent
Guthion	---	---
Malathion	1	3.3
OMPA	---	Present in carrier solvent
Parathion	1	3.9
Phosdrin	1	0.8
Ronnel	1	2.9
Systox	1	1.9
TEPP	1	Present in carrier solvent
Sevin	1	1.7

The retention values of Tables V and VI revealed that the column temperature 247°C, was ideal for separation of chlorinated organic pesticides, eg. DDT, Endrin, and Methoxychlor; however, it was too high for good separation of organic phosphate pesticides. To correct this, temperature programming was used. Briefly, temperature programming is a technique of linearly increasing the temperature of the gas chromatographic column during the analysis. In the analysis of pesticides, the temperature was programmed between 160 and 260°C and 20° per minute. Temperature programming gave better resolution and decreased the time of analysis. The retention time information obtained under isothermal conditions could be used to calculate retention time in a temperature programmed scheme according to Gidding (7).

In order to evaluate the temperature programmed gas chromatography, field samples were analyzed. Though the samples were cleaned by passage through a column of activated aluminum silicate, sufficient elutable extraneous matter remained. Figure 2 is typical of the chromatograms obtained from the analysis of field samples. The sample chromatographed was an aliquot of an extract obtained from the sediment in Moses Lake near the confluence of Crab Creek in Grant County, Washington. The extraneous matter prevented an interpretation of the chromatogram.

It is apparent if gas chromatography were to be used for the investigation of field samples, a detector more specific than the hydrogen flame detector would have to be employed. One such detector is the electron-capture attachment. A Wilkens Aerograph Hy-Fi chromatograph with electron-capture detector having a tritium source was used. Figure 3 is a chromatogram of an aliquot of the Moses Lake sediment extract. A 5 ft. long, 1/8 inch pyrex glass column packed with SE 30 silicone oil on 60/80 mesh chromosorb was used. The column temperature was maintained at 200° C. The carrier gas (nitrogen) flow rate was 30 mls./min. An attenuation of one and a cell potential of 90 volts was used. The chromatogram (Figure 3) shows the presence of fifteen components as compared with eighteen components sensed by the hydrogen flame detector. Even though the electron capture detection reduced the number of components, it is impossible to clearly discern those peaks attributed to pesticides. An advantage of the electron-capture detector is that it can be operated at full sensitivity for it was insensitive to column bleed-off. With the hydrogen flame detector, an attenuation of 2 x 100 must be used to nullify the effect of column bleed off. Therefore, in pesticide analysis, the electron-capture detector is in effect, 200 times more sensitive than the hydrogen flame detector.

The third detector evaluated was a microcoulometric detector. For the microcoulometric analysis of the Moses Lake sediment extract, an aliquot was injected into a Beckman temperature programmer coupled with a Dohrmann microcoulometric detector. A column 5 ft. long of 1/4 inch diameter aluminum tubing packed with 5% Dow 200 silicone oil on 60/80 mesh acid washed Chromosorb P was used. The temperature was programmed between 200 and 230°C at 6 degrees per

7. Giddings, J. C., "The Science of Programmed Temperature Gas Chromatography," Facts and Methods for Scientific Research, F & M Corp. 3, 2 Summer issue 1962.

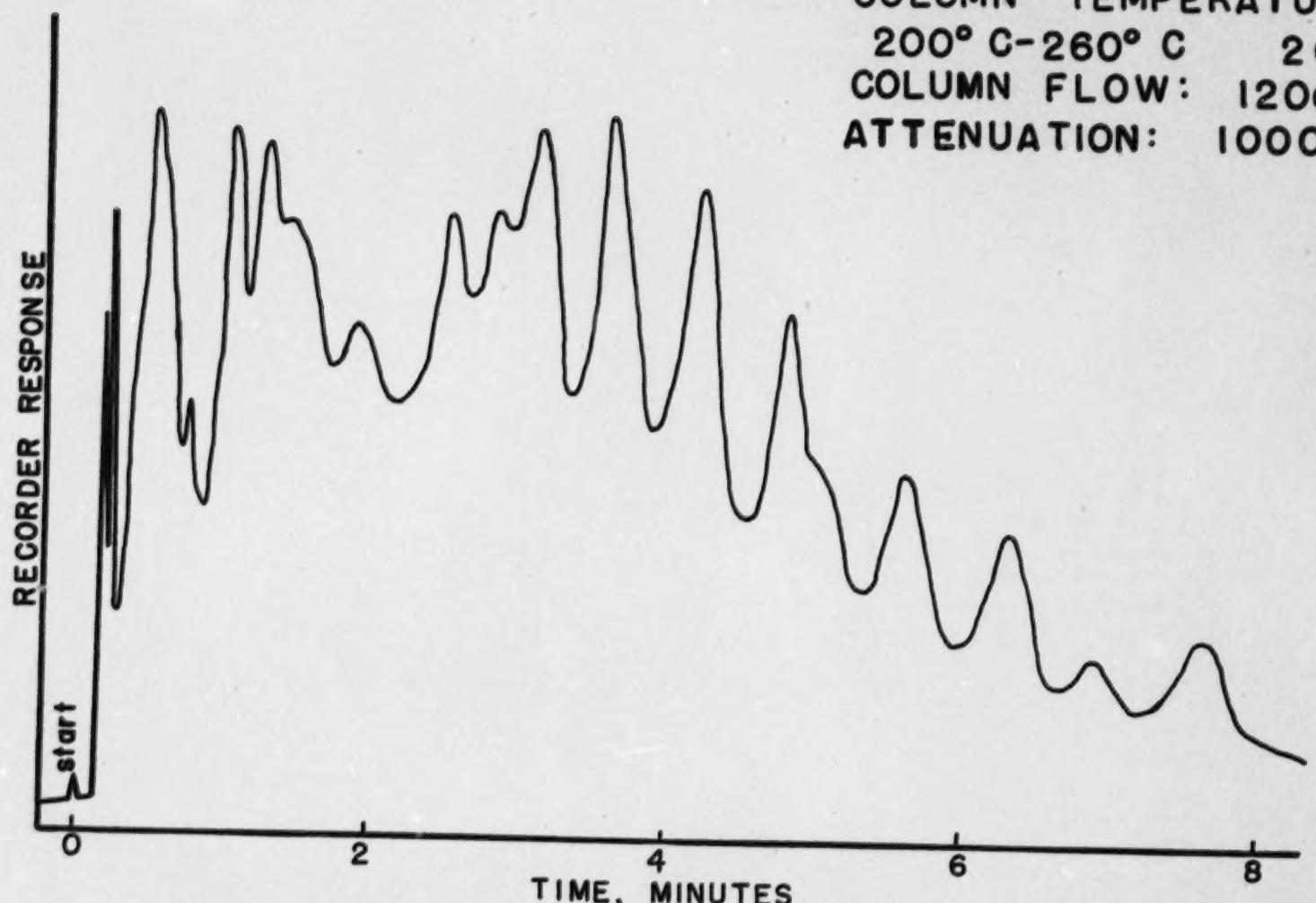

Fig.2 CHROMATOGRAM OF MOSES LAKE-2 USING HYDROGEN FLAME DETECTOR

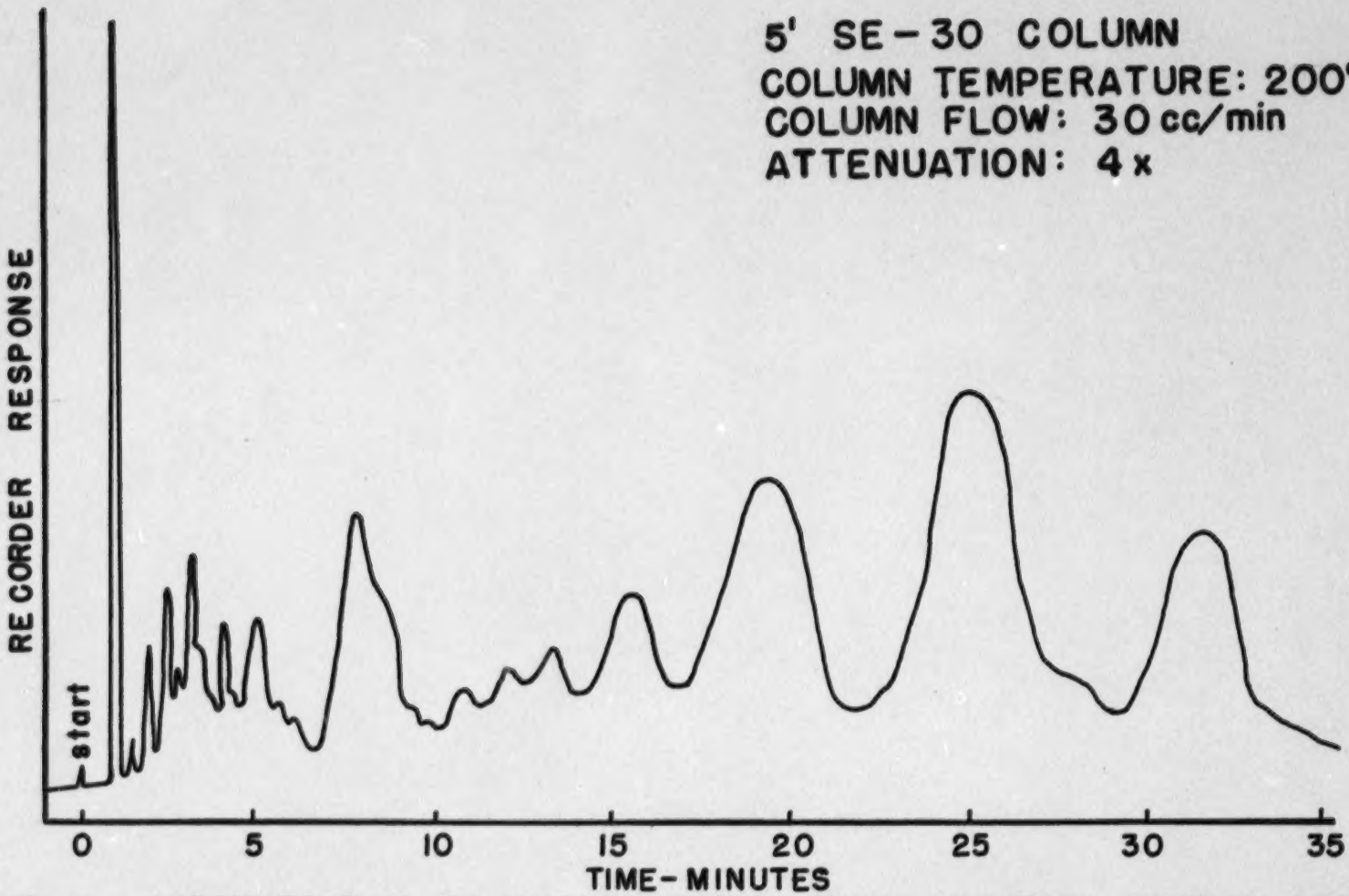

Fig. 3 CHROMATOGRAM OF MOSES LAKE-2 USING ELECTRON CAPTURE DETECTOR

minute. The microcoulometer was operated at maximum sensitivity (512 ohms.), with a damping position 4, and a bias of 250 millivolts. Figure 4 is the chromatogram obtained from the extract of Moses Lake sediment. The retention time of the peaks corresponds to the three isomers of DDT. The presence of DDT was later confirmed by thin-layer chromatographic methods.

Specificity required for the analysis of pesticides in extracts from field samples can be analyzed best by a microcoulometric detector. It should be noted that the electron-capture detector is 10 to 100 times as sensitive as the microcoulometric detector for the detection of chlorinated organic compounds. Thus for extremely clean extracts it may be more desirable to use a chromatograph equipped with an electron-capture detector.

As part of a study of the effects of irrigation water on the water quality of the surface and subsurface water in the Columbia Basin, samples were obtained for pesticide analysis during the 1961 and 1962 irrigation seasons. Water samples were sent through a carbon filter for pesticide removal. The pesticide was deadsorbed from the activated carbon with Skellysolve. The solvent was removed, the residue dried then made up to a known volume. A 10 microliter aliquot was chromatographed using a silicone oil - epon column programmed at 160° to 250° at 20 degrees per minute. A microcoulometric detector with a chloride titration cell was used. The coulometer was operated at maximum sensitive damped to 4 units, and had a bias of +250 Millivolts.

The information obtained from samples collected during the 1961 irrigation season is shown in Table VII while that tabulated for the 1962 season is shown in Table VIII.

Because a sulfur-titration cell was not available for use with the microcoulometer, organic phosphate pesticides containing sulfur could not be detected at that time; however, the data in Tables VII and VIII indicate that return flows, surface drainage and shallow subsurface flows from treated croplands contain minute quantities of chlorinated pesticides. Pesticides have been detected in higher concentrations in the bottom sediments of streams and reservoirs than from the moving water. This can be attributed to the presence of pesticide-laden sediments transported from treated lands.

Hydrolysis of Organic Phosphate Insecticides

Organic phosphates because of their chemical structure, are subject to hydrolysis. The factors affecting hydrolysis in a turbid stream or in irrigation return flow are not known. A study was undertaken to determine the effect of suspended soil on the hydrolysis of three organic phosphate pesticides. Malathion, systox and phosdrin (typical representatives of the phosphorodithioates, phosphorothioates and phosphonate pesticides, respectively) were studied. The effects were investigated of a soil suspension of various types of soils on the rate of removal of the pesticide from the solution.

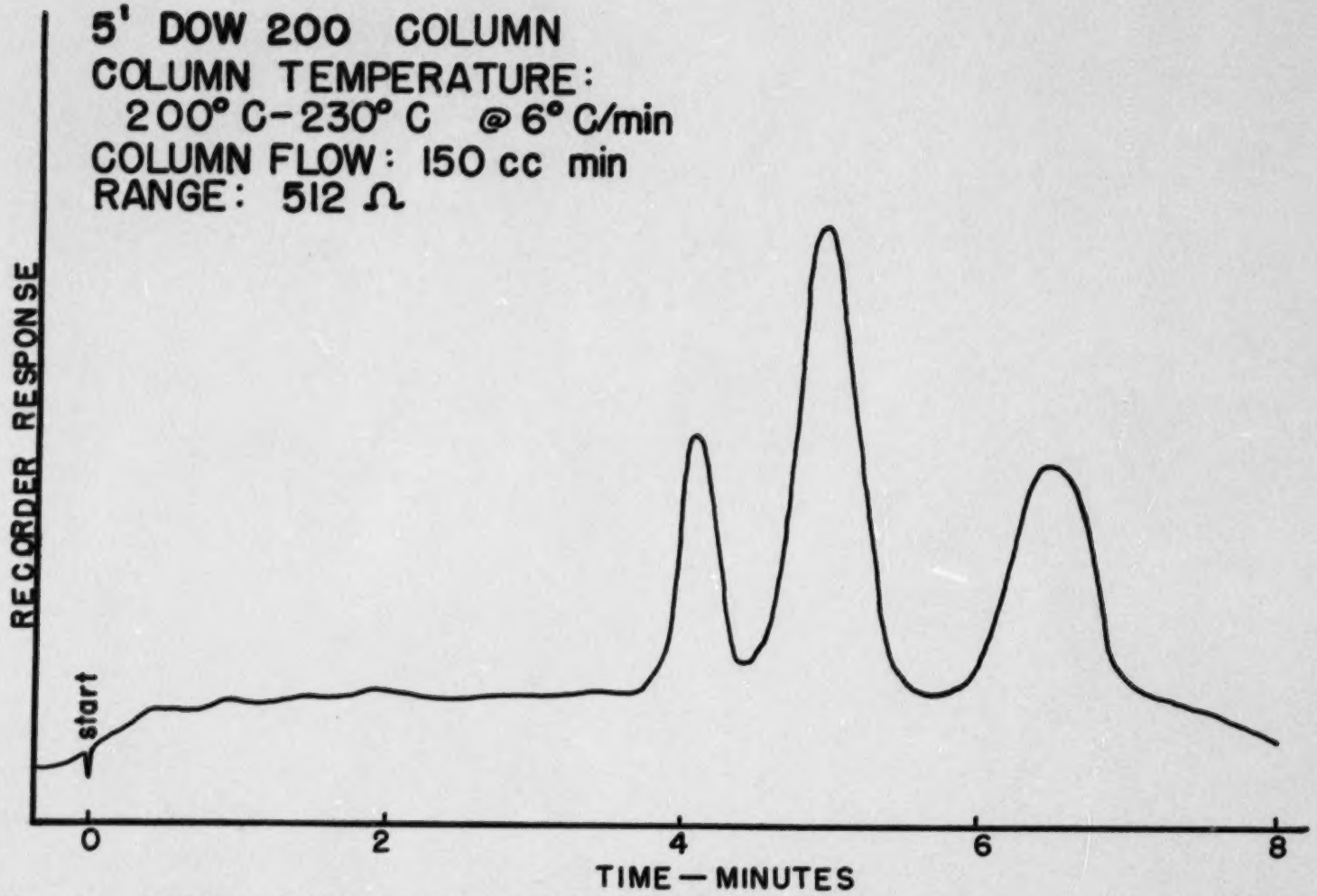

Fig. 4 CHROMATOGRAM OF MOSES LAKE-2 USING MICROCOULOMETRIC DETECTOR

TABLE VII

PESTICIDES IN COLUMBIA BASIN WATER SAMPLES DURING THE 1961 IRRIGATION SEASON

Type/Location	Pesticide	Concentration ng/l
Irrigation return flow Quincy area	Aldrin	0.25
	DDT	1.03
Irrigation return flow Moses Lake area	DDT	4.0
	2,4-D isopropyl ester	18.0
Irrigation water-West Canal near Quincy	DDT	0.7
Irrigation return flow Quincy area	Aldrin	0.1
	DDT	trace
Irrigation return flow Moses Lake area	DDT	16
	2,4-D isopropyl ester	3
Irrigation return flow at Evergreen Pumping plant	DDT	0.5
	2,4-D isopropyl ester	0.7
Irrigation return flow Winchester Waste Way at U. S. Highway 10	DDT	0.1
	2,4-D isopropyl ester	0.4
Irrigation Return flow Crab Creek at Smyrna	DDT	2.2
	2,4-D isopropyl ester	4.0
Well - from farm in the Moses Lake area	DDT	0.03
Well - from U.S.B.R. west of Moses Lake	DDT	0.05
Irrigation water in Quincy area	DDT	0.1
	2,4-D isopropyl ester	0.3
Irrigation Return flow Moses Lake area	2,4-D isopropyl ester	1.1
	DDT	0.5
Main Irrigation Canal below Long Lake Dam	2,4-D isopropyl ester	0.5
	DDT	0.1
Scooteney Lake Outlet	DDT	1.0
Potholes Irrigation Canal South of Othello	2,4-D butyl ester	0.7
Mouth of the Wenatchee River	DDT	1.5

TABLE VII, Continued

Type/Location	Pesticide	Concentration ng/l
Outlet of the Scooteney Reservoir	DDT	0.8
Irrigation Water West Canal	DDT	0.06
Irrigation Return Flow in the Ringold Springs area	DDT	0.05
	2,4-D isopropyl ester	0.8
	Endrin	0.4
Irrigation Return flow Ringold Springs area	Aldrin	2.0
Irrigation Return flow Moses Lake area	2,4-D butyl ester	4
	Endrin	57
Mouth of the Entiat River	DDT	4.0
Bottom sediment from the Wenatchee River	DDT	56 µg/Kg.
Bottom sediment from the Entiat River	DDT	144 µg/Kg.
	DDD	95 µg/Kg.

TABLE VIII

PESTICIDES IN WATER SAMPLES FROM THE COLUMBIA BASIN - 1962 - SEASON

Type/Location	Pesticide	Concentration ng/l
Irrigation Canal in West of Quincy	2,4-D isopropyl ester	Trace
	Aldrin	1.6
Irrigation return flow ditch - south of Quincy	2,4-D isopropyl ester	Trace
	Aldrin	0.8
	DDT	0.8
Return flow to Evergreen Lake	2,4-D isopropyl ester	1.7
Return flow - Winchester Wasteway at U. S. Highway 10	2,4-D isoproply ester	2.0
	Aldrin	0.4
Columbia River 1.2 mi. So. of Wenatchee	2,4-D isopropyl ester	1.5
Entiat River, 1 mile from mouth	Aldrin	0.04
	DDT	0.8
Return flow - Winchester Wasteway at U. S. Highway 10	Aldrin	0.2
Irrigation return flow ditch - south of Quincy	2,4-D isopropyl ester	1.8
	Aldrin	0.8
	DDT	0.9
Return flow - Winchester Wasteway at U. S. Highway 10	2,4-D isopropyl ester	0.1
	Aldrin	0.1
	DDT	0.2
	DDD	0.4
Wenatchee River - at confluence with the Columbia River	DDT	0.6
	DDD	0.4
Bottom sediment from Evergreen Lake - west end	Aldrin	0.9 ug/Kg.
Bottom sediment from the middle of Evergreen Lake	Aldrin	1.2 ug/Kg.
	DDT	1.5 ug/Kg.
	DDD	6.0 ug/Kg.
Bottom Sediment from inlet chanel to Coffin Lake	Aldrin	2.3
	DDT	0.7
	DDD	0.6

TABLE VIII Continued

Type/Location	Pesticide	Concentration ug/Kg.
Bottom sediment from Coffin Lake - 200 yd. from the inlet	Aldrin	1.7
	DDT	1.7
	DDD	2.5
Bottom sediment from Crab Creek at State Highway 11G	DDT	9.6
Bottom sediment from Moses Lake off Parker Horn	DDT	4.8

The experimental procedure used follows: 120 milligrams of pesticide was added to each of two carboys; one containing 4 liters of distilled water, the other containing 454 grams of soil in 4 liters of water. The two solutions were continuously mixed to insure an even distribution of pesticide in solution in addition to keeping the soil in suspension. A 400 ml. aliquot of the solution was removed at 12-hour intervals when malathion or systox was studied and at 4-hour intervals when phosdrin was investigated. The aliquots were extracted as follows:

> A 400 ml. aliquot of the aqueous silt solution was centrifuged. The liquid portion was transferred to a two-liter separatory funnel and extracted repetitively with three 50 ml. portions of a petroleum ether - ethyl ether mixture. The water ether phases were mixed by vigorous shaking for 30 seconds and allowed to separate. The combined extracts were filtered through anhydrous sodium sulfate to remove all traces of water. The filtrate was collected in a 500 ml. round bottom flask and evaporated to almost dryness by rotatory evaporation at 35°C under a slight vacumn. The concentrate was transferred to a 10 ml. vial. Three 2.5 ml portions of petroleum ether was used to facilitate the transfer. The concentrate was evaporated to dryness with a stream of cool dried air. One-half ml. portions of petroleum ether were used to rinse down the walls of the vial. The residues were refrigerated until ready for analysis. At the time of analysis, the residue was taken up in a measured amount (1 ml. or 0.1 ml of benzene). A programming gas chromatograph with a microcoulometer-sulfur titration cell detector was used. Table IX lists varieties and types of soils used in this study.

Table IX

SOME CHARACTERISTICS OF SOILS USED

Series	Type	pH range	Organic Matter	Major exchangeable ions
Colville	silty loam	8-8.5	10-20%	Ca^{+2}
Hezel	sandy loam	7-8	1%	Ca^{+2}
Muck	peat	4-5	80%	H^{+}, Al^{+3}
Puyallup	sandy loam	5.5-6.5	4-6%	H^{+}, Al^{+3}, Ca^{+2}
Walla Walla	silty loam	5.8-6.5	3%	Ca^{+2}
Wantum	saline-sandy loam	9	2-3%	Na^{+}

Figure 5 and 6 represent graphically the removal characteristics by each of the six soils suspensions of malathion. Note that less than 10% of malathion is hydrolyzed in distilled water after 72 hours. All soil suspensions took up malathion immediately. It is assumed that this can be attributed in part to the adsorption of malathion on the soil surface. No correlation can be found

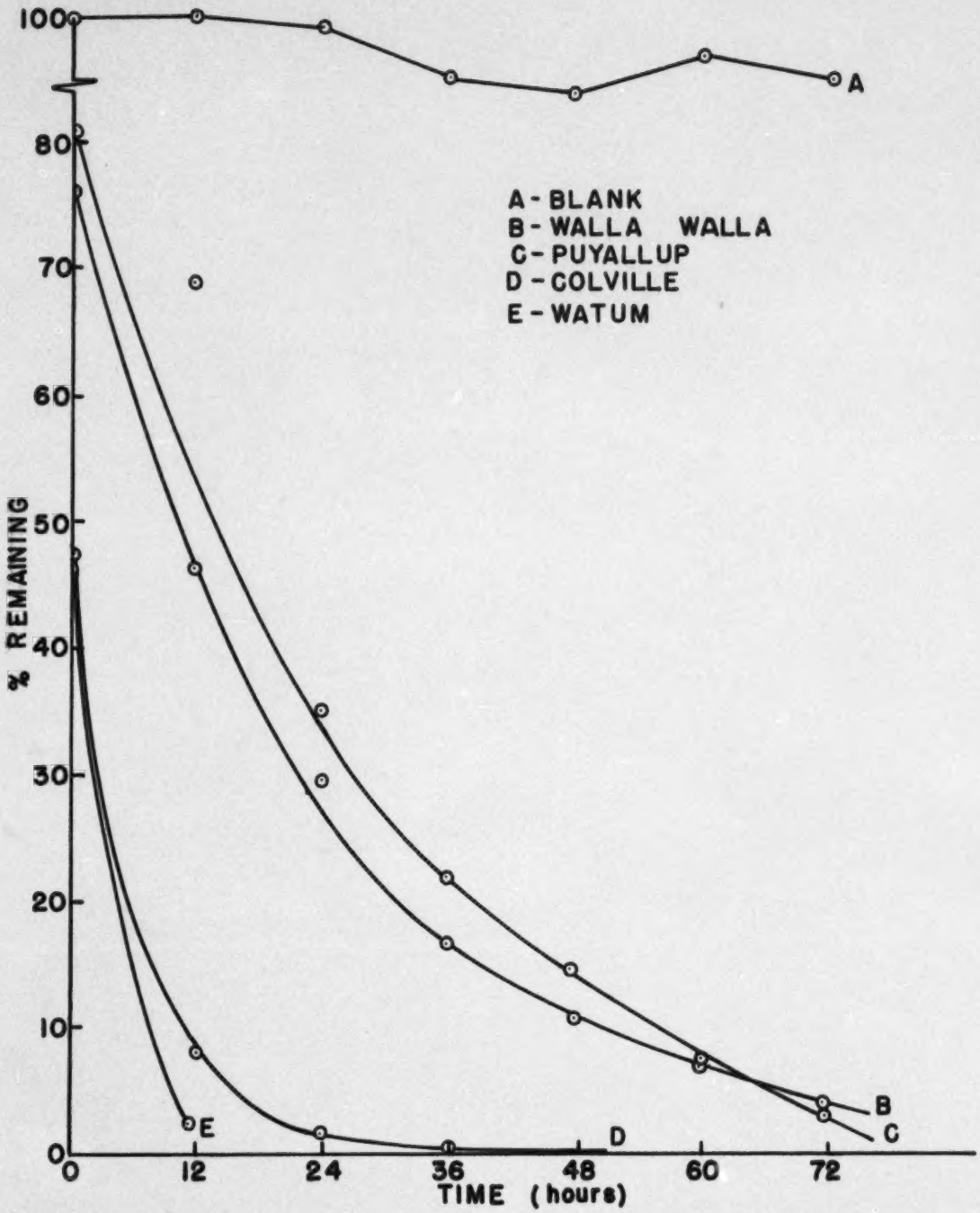

Fig. 5 REMOVAL RATE OF MALATHION FROM SOIL SUSPENSIONS

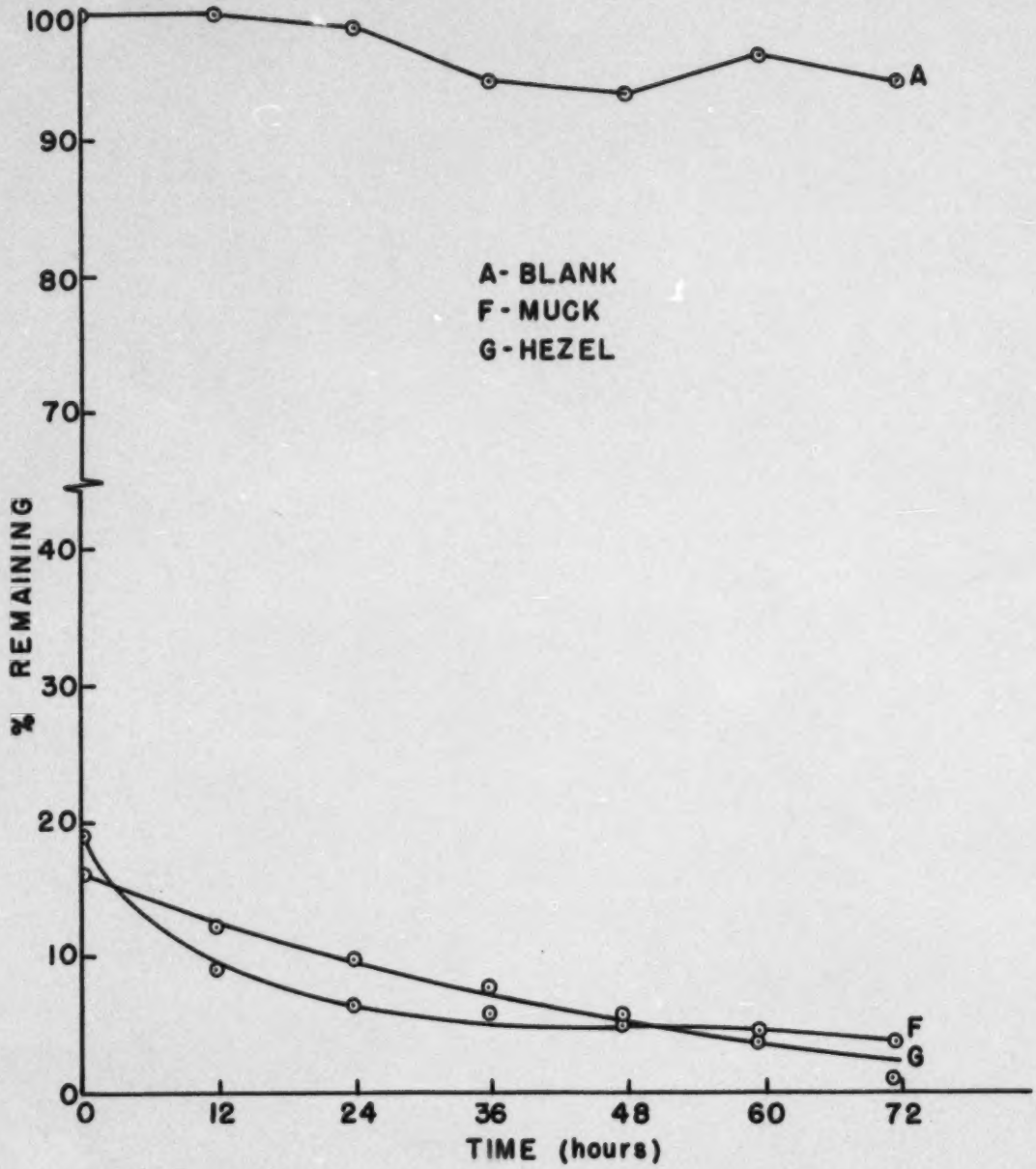

Fig. 6 REMOVAL RATE OF MALATHION FROM SOIL SUSPENSIONS

between the adsorption capacity and soil type. Immediate uptake is probably a combination of physical and chemical action of the soil. The rate of hydrolysis of malathion in suspension of Muck and Hezel soils approximates that of malathion in distilled water; this rate, however, was not the same for the remaining soil suspensions. Alkaline soils such as Colville and Wantum (pH range above 8) showed the greatest increase in the rate of hydrolysis. Other generalizations could not be based on the graphical representation of the data. It is apparent that the data of a complete chemical analysis of each soil in addition to certain physical measurements must be available to correlate the magnitude of the immediate uptake of malathion to each type of soil. Data regarding the exchangeable soil ions would be required to explain the effect of the soil on the hydrolysis of malathion.

Systox hydrolyzed at a greater rate than malathion. After 60 hours 24% of the systox was lost through hydrolysis, according to Figure 7. As in the malathion studies, an immediate uptake of systox by the suspended soil occurred. Note that the order of the immediate soil uptake of systox was similar to that obtained for malathion. The four soil suspensions investigated had little effect on the hydrolysis rate of systox beyond that of distilled water.

The hydrolysis rate of phosdrin in distilled water was very rapid. In 8 hours, 71% of this pesticide was hydrolyzed. Suspensions of Colville and of Walla Walla soils were used. It was found that there was an immediate uptake of phosdrin by the soil in suspension; however, the soluble and suspended soil constituents had little effect on the rate of hydrolysis beyond that which can be expected from distilled water according to Figure 8.

It can be concluded from the hydrolysis of organic phosphate pesticides study that:

1. Phosphorates hydrolyze more readily but not as completely as the phosphorothioates.

2. The soluble constituents of six soils tested had little effect on the hydrolysis of phosphonate and phosphorothioate pesticides. With suspensions of four of the six types of soil, the hydrolysis of the phosphorodithioate pesticide was greater than that of distilled water.

3. There is an immediate uptake of organic phosphate pesticide when placed in a soil suspension.

A preliminary study was undertaken to determine the uptake of systox by algae. The species used in the study was Chlorella. Systox was added to a buffered growth medium containing systox. After three days the algal cells were harvested, washed and the cellular matter removed. The algae had incorporated 10% of the toxic substance either as sytox or a toxic metabolite in its protoplasm. The quantity of cholinesterase inhibiting substance was determined by the enzymatic method.

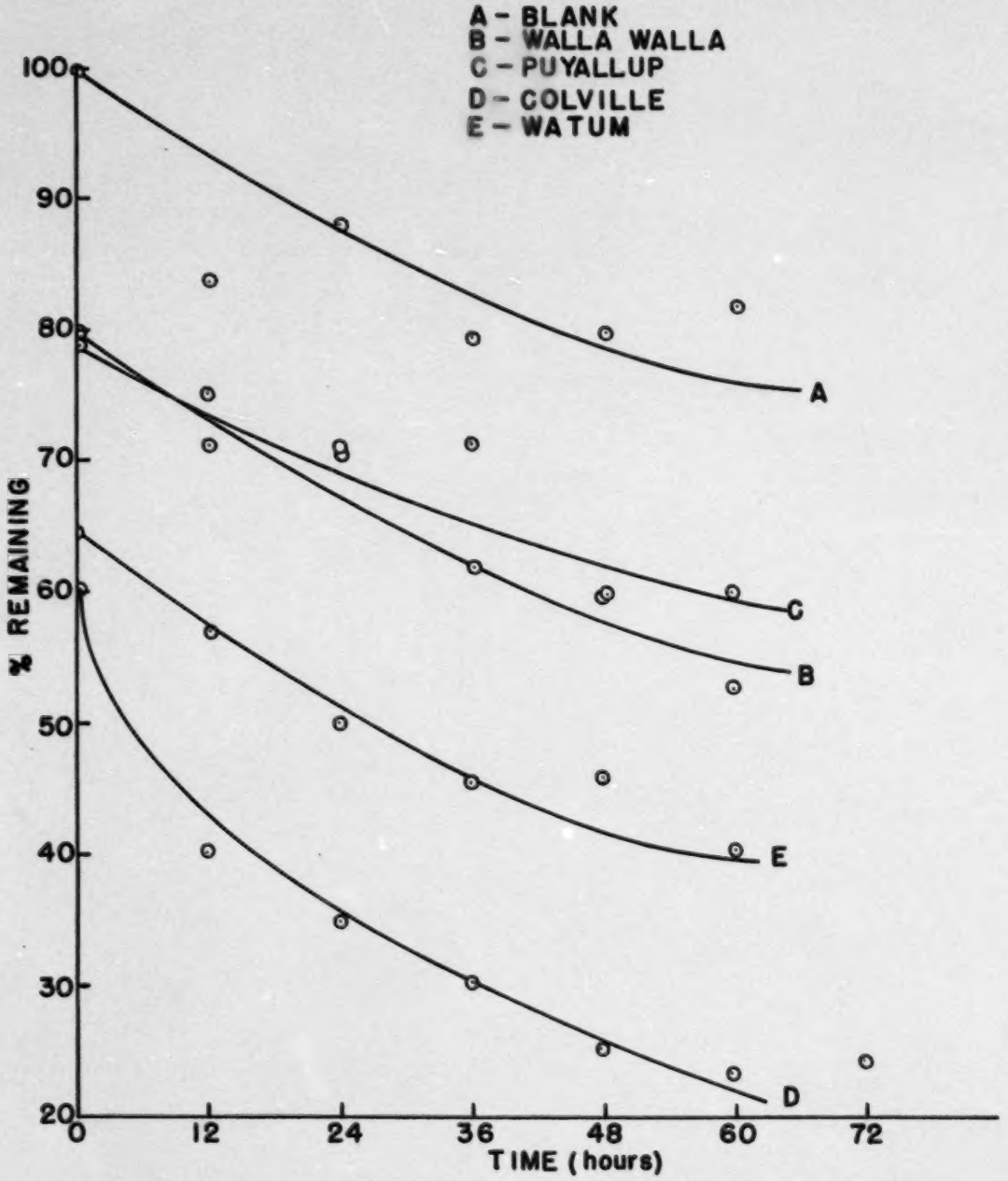

Fig. 7 REMOVAL RATE OF SYSTOX FROM SOIL SUSPENSIONS

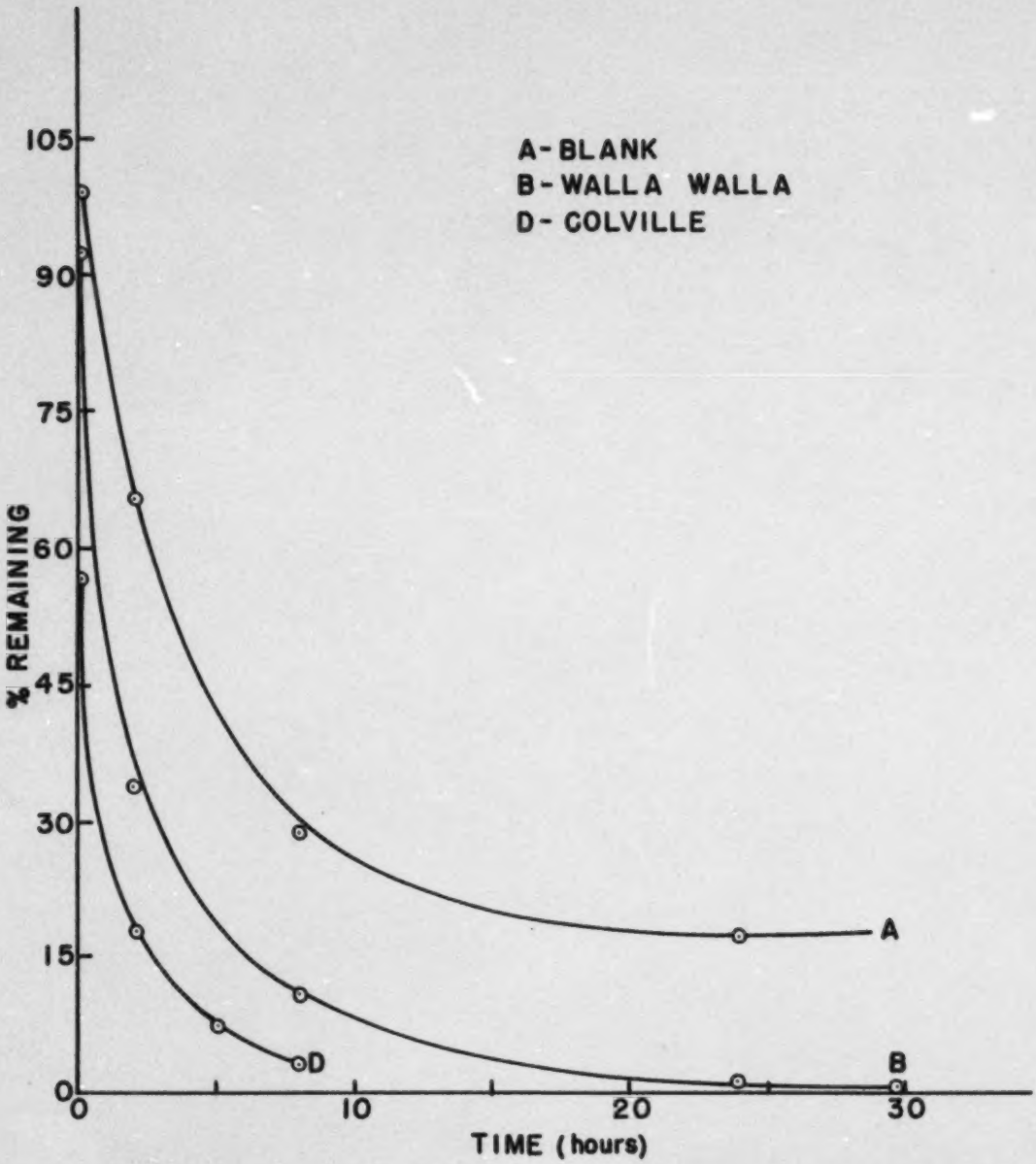

Fig. 8 REMOVAL RATE OF PHOSDRIN FROM SOIL SUSPENSIONS

A cooperative study was undertaken with the Irrigation Experiment Station and the Washington Agricultural Experiment Station of Washington State University to assess the pesticide distribution on an experimental plot. Samples are being extracted and will be analyzed late in 1963. The financial support for the completion of this aspect of the grant is being carried out from Washington State University funds. A paper based on the information obtained from the cooperative study will be forthcoming.

The information obtained in studies supported by Grant WP-00215 has been presented before technical meetings and/or as published in appropriate journals. The following is a list of such papers.

1. Hindin E., and Dunstan, G. H., "Analysis of Synthetic Organic Pesticides by Chromatography, 16th Annual Northwest Regional Meeting of the American Chemical Society, June, 1961.

2. Hindin, E., Hatten, M. J., May, D. S., Skrinde, R. T., and Dunstan, G. H. "Analysis of Synthetic Organic Pesticides in Water," Journal American Water Works Association 54, 1, 88, Jan. 1962.

3. Hindin, E., and Dunstan, G. H., "Analysis of Pesticides in Water," Proceedings of the 1961 British Conference on Insecticides and Herbicides.

4. Hindin, E., May, D. S., and Dunstan, G. H., "Analysis of Organic Pesticides by Gas Chromatography." Paper given at and will appear in the Proceedings of the 18th Annual Purdue Industrial Waste Conference, May, 1963.

5. May, D. S., Hindin, E., and Dunstan, G. H., "Collection and Analysis of Synthetic Organic Pesticides from Surface and Ground Water Supplies," To appear in Residue Review Vol. 9, Spring of 1964. This paper was presented at the 47th Annual Meeting, Entomological Society of America, Pacific Branch - Gearhart, Oregon. June 1963.

APPENDIX A

References of the Abstracted Publications

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II. Methods of Sample "Clean up"

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